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**14. ABSTRACT**

Current osteoporosis therapies are able to treat the symptoms of osteoporosis, however little progress has been made toward understanding and addressing the underlying mechanisms contributing to age-related bone loss, or the ability to adapt to mechanical loading (exercise). Degeneration in peripheral nerve function with age may be one of these mechanisms, as neuropeptides affect the function of osteoblasts and osteoclasts in vitro, and nerve deactivation causes bone loss in vivo. This research investigates mechanisms by which peripheral sensory nerves influence bone maintenance and mechanotransduction using capsaicin-injected mice as a model of decreased peripheral sensory nerve function. We hypothesize that decreased sensory nerve function will result in increased functional adaptation of bone. In Aim 1 we investigated the relationship between peripheral sensory nerve function and bone structure. We found that capsaicin treatment resulted in a small but statistically significant decrease in trabecular and cortical bone structure. In Aim 2 we will determine the bone adaptation response of capsaicin- and vehicle-treated mice to increased mechanical loading. We hypothesize that capsaicin-treated mice will have an increased bone adaptation response to mechanical loading. The proposed research will establish the role of peripheral sensory nerves in age-related decreases in bone's ability to adapt to exercise. These studies may lead to novel therapies aimed at preserving healthy bone turnover with age. This research will be the basis for future studies investigating the interaction of peripheral nerves and bone, and peripheral nerve function as a potential mechanism of age-related bone loss.

**15. SUBJECT TERMS**
Peripheral sensory nerves, osteoporosis, bone structure, trabecular, cortical, mechanical loading, bone adaptation

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**INTRODUCTION:** Osteoporosis is a major public health concern for an ever-growing aging population, affecting over 44 million Americans [1]. Osteoporotic fractures are associated with morbidity, increased mortality, and a general decrease in quality of life. While current pharmacological therapies are useful for treating symptoms of osteoporosis, little progress has been made toward understanding and addressing the *underlying mechanisms leading to age-related bone loss*. Diminished innervation of bone with age may be one of these mechanisms, as peripheral nerve function has been shown to affect bone metabolism both *in vitro* [2-7] and *in vivo* [8, 9]. The proposed research investigates the role of peripheral sensory nerve function on bone maintenance and mechanotransduction using capsaicin-treated mice as a model of decreased peripheral nerve function. We hypothesized that decreased peripheral nerve function will result in an *increased* functional adaptation response of bone, due to decreased negative feedback on osteoblast and osteoclast function. To test this hypothesis, we proposed two specific aims. The first aim will determine the effects of neonatal capsaicin treatment on bone structure and metabolism compared to vehicle-injected mice. The second aim will determine the bone adaptation and peripheral nerve response of capsaicin- and vehicle-injected mice to increased mechanical loading. This research will establish the role of peripheral nerves in bone metabolism and mechanotransduction, and may lead to novel therapies aimed at preserving healthy bone turnover with age.

**BODY:**

**Approved Statement of Work:**

**Aim 1.** *Determine the effects of neonatal capsaicin treatment on bone structure and metabolism, and neuropeptide concentrations in bone compared to vehicle-injected mice* (months 1-12):

1a. Institutional approval of animal use protocols (*this will be done before the funding period begins)
1b. Capsaicin or vehicle treatment of neonatal mice (months 1-3)
1c. Hot plate analgesia meter testing of mice to determine sensory threshold (months 2-4)
1d. Micro-computed tomography of mouse bones (months 2-6)
1e. ELISA analysis of neuropeptides in bone (months 2-10)
1f. Multiplex analysis of bone biomarkers (months 2-10)
1g. Embedding/cutting/imaging/analysis of bones for dynamic histomorphometry (months 4-12)

**Aim 2.** *Determine the bone adaptation and peripheral nerve response of capsaicin- and vehicle-injected mice to increased mechanical loading* (months 6-18):

2a. Strain gage analysis of bone strain during tibial compression (months 6-7)
2b. Capsaicin or vehicle treatment of neonatal mice (months 6-8)
2c. Tibial compression of capsaicin- and vehicle-injected mice (months 8-10)
2d. Micro-computed tomography of mouse bones (months 9-12)
2e. ELISA analysis of neuropeptides in bone (months 10-16)
2f. Multiplex analysis of bone biomarkers (months 10-16)
2g. Embedding/cutting/imaging/analysis of bones for dynamic histomorphometry (months 10-18)

As of the date of this report (28 October, 2013), all items for Aim 1 have been accomplished. The only notable change was the use of ELISA rather than Multiplex analysis for item 1f. We chose to analyze established serum biomarkers of bone metabolism (P1NP and CTX-I) using ELISA rather than the less established markers quantified by the multiplex kits. We are currently working to complete tasks 2a and 2b, after which the remainder of Aim 2 will be accomplishable.

*Capsaicin or vehicle treatment of neonatal mice (1b):*

A total of 42 male and female C57BL/6 neonatal mice were used in this study (Harlan Laboratories, Indianapolis, IN). Neonatal capsaicin treatment was performed as previously described [10]. Briefly, neonatal mice were given subcutaneous injections of capsaicin (50 mg/kg) or vehicle (10% ethanol, 10% Tween 80 in isotonic saline) on day 2 and 5 after birth (n = 21 vehicle, 21 capsaicin). Following capsaicin or vehicle treatment, neonatal mice were returned to normal cage activity until weaning (28 days). Mice were sacrificed 4, 8, or 12 weeks after birth (n = 7 mice for each age/treatment group). We selected these time points to observe developmental changes from weaning until skeletal maturity, and to account for any "recovery" from sensory nerve inactivation by 12 weeks of age. Mice were weighed 1-2 times per week from birth until sacrifice.

Capsaicin treatment did not have an effect on the body weights of male mice; there was no difference in body weight between capsaicin- and vehicle-treated male mice for any time point from weaning until 12 weeks (Fig. 1). However, capsaicin treatment significantly affected female mice. Female mice treated with capsaicin had 6-13% lower body weights than vehicle-treated female mice from weaning until 8 weeks of age (Fig. 1; $p < 0.05$ for all time points). There were no significant differences in body weights for capsaicin- and vehicle-treated female mice from 8 to 12 weeks of age.

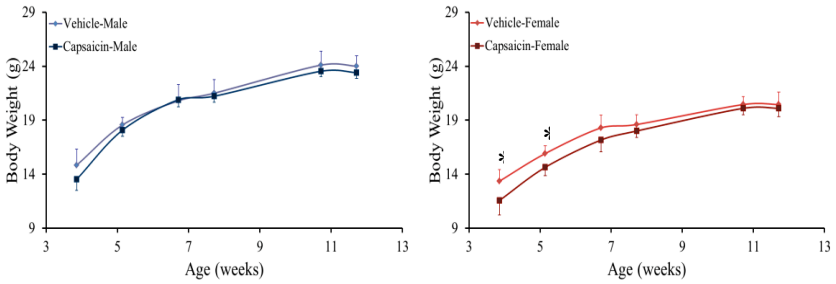


Fig. 1. Female mice treated with capsaicin had lower body weight than vehicle-treated female mice from weaning until day 47. * $p < 0.05$.

*Hot plate analgesia meter testing of mice to determine sensory threshold (1c):*

Capsaicin- and vehicle-treated mice were subjected to hot-plate analgesia testing at 4, 8, and 12 weeks of age to determine response time to a constant thermal stimulus of 55 °C as previously described [11]. Mice were placed on a hot-plate (LE 7406, Coulborn Instruments, Whitehall, PA) and removed after indication of discomfort, determined as twitching or licking of a hind limb or jumping, or after a maximum of 30 seconds, and the latency time of the response was recorded. The experiment was performed twice at each time point and the latency times averaged for each mouse.

Mice treated with capsaicin had significantly longer latency times when exposed to the constant thermal stimulus than vehicle-treated mice (Fig. 2; $p = 0.0003$). At 8 weeks of age, the average latency time of capsaicin-treated mice was 39% longer than that of vehicle-treated mice ($p = 0.00022$), while at 12 weeks of age the latency time was 42% longer for capsaicin-treated mice ($p = 0.037$). This confirms that mice treated with capsaicin as neonates had decreased peripheral sensory nerve function, which persisted until at least 12 weeks of age.

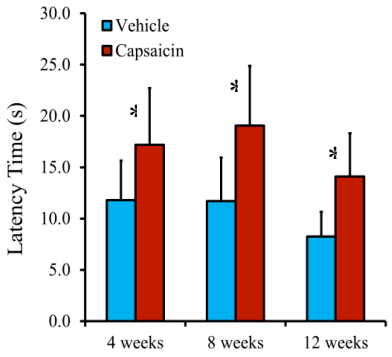


Fig. 2. Capsaicin-treated mice had significantly longer latency times than vehicle-treated mice when exposed to a constant thermal stimulus, consistent with decreased peripheral sensory nerve function. * $p < 0.05$.

*Micro-computed tomography analysis of bone structure (1d):*

Right tibias, right femurs, and L5 vertebrae were removed post mortem and preserved in 70% ethanol. Bones were scanned using micro-computed tomography (SCANCO, µCT 35, Bassersdorf, Switzerland); images were acquired at 6 µm nominal voxel size (energy=55 kVp, intensity=114 µA, integration time = 900 ms). Trabecular bone was analyzed at the metaphysis and epiphysis of the distal femur and at the L5 vertebral body using manually drawn contours inside the cortical shell on two-dimensional slices. The metaphysis was defined by a 900 µm thick volume of interest beginning below the middle break of the growth plate. Trabecular bone volume per total volume (BV/TV), trabecular number, trabecular thickness (Tb.Th), and trabecular separation were determined using the manufacturer's 3-D analysis tools. Cortical bone was analyzed at the mid-diaphysis of the tibia and femur, using a 240 µm thick volume of interest centered at the measured midpoint of each bone. Bone area (B.Ar), medullary area (M.Ar), total cross-sectional area (Tt.Ar), and cortical thickness (Ct.Th) were determined using the manufacturer's 3-D analysis tools.

Capsaicin treatment significantly decreased bone structure parameters in trabecular bone of the mice (Table 1). There was a main effect of capsaicin treatment on Tb.Th at the femoral metaphysis ($p = 0.0002$). For example, female capsaicin-treated mice had 5.2-9.5% lower Tb.Th than vehicle-treated female mice at this location at each time point ($p = 0.0082$-$0.049$). However, there was no effect of capsaicin treatment on BV/TV or Tb.Sp at the femoral metaphysis. At the femoral epiphysis, we observed a significant treatment*sex interaction for Tb.Th. ($p = 0.026$). We also found a significant treatment*sex*age interaction for BV/TV. For example, female capsaicin-treated mice had 8.7% lower BV/TV at the femoral epiphysis than vehicle-treated

female mice at 8 weeks, whereas male mice had similar BV/TV at this time point (p = 0.015). At 12 weeks, female capsaicin-treated mice actually demonstrated 5.7% higher BV/TV than vehicle-treated mice.

Table 1. Distal femoral metaphysis trabecular bone structural parameters.

| | *BV/TV (%)* | *Tb.N (1/mm)* | *Tb.Th (μm)* | *Tb.Sp (μm)* |
|---|---|---|---|---|
| **Vehicle-treated** | | | | |
| 4 week | | | | |
| M | 12.7 ± 1.7 | 5.9 ± 0.6 | 30.4 ± 2.0 | 170 ± 18 |
| F | 10.5 ± 1.2 | 5.4 ± 0.6 | 26.9 ± 0.3 | 192 ± 24 |
| 8 weeks | | | | |
| M | 10.4 ± 1.0 | 5.1 ± 0.1 | 31.4 ± 1.1 | 194 ± 5.7 |
| F | 10.6 ± 2.2 | 5.2 ± 0.5 | 31.5 ± 1.6 | 193 ± 19 |
| 12 weeks | | | | |
| M | 11.7 ± 1.5 | 4.9 ± 0.2 | 38.7 ± 2.0 | 197 ± 9.8 |
| F | 10.2 ± 1.9 | 4.2 ± 0.3 | 38.6 ± 0.3 | 242 ± 18 |
| **Capsaicin-treated** | | | | |
| 4 weeks | | | | |
| M | 10.1 ± 3.2 | 5.6 ± 0.8 | 26.8 ± 2.3[a] | 180 ± 23 |
| F | 9.56 ± 2.0 | 5.3 ± 0.6 | 25.5 ± 0.8[a] | 192 ± 24 |
| 8 weeks | | | | |
| M | 11.1 ± 1.2 | 5.6 ± 0.2 | 31.6 ± 1.2 | 176 ± 7.7 |
| F | 9.24 ± 0.75 | 5.2 ± 0.2 | 28.5 ± 0.9[a] | 190 ± 7.7 |
| 12 weeks | | | | |
| M | 11.9 ± 0.60 | 5.1 ± 0.2 | 37.8 ± 0.6 | 193 ± 9.1 |
| F | 8.88 ± 0.30 | 4.3 ± 0.2 | 36.3 ± 1.4[a] | 232 ± 12 |
| | | | | |

Trabecular bone parameters measured using microCT. All data are expressed as mean ± SD.
[a] Significant difference between capsaicin and vehicle-treated mice, p < 0.05.

Capsaicin treatment reduced cortical bone structure parameters as well. There was a main effect of capsaicin treatment on femur length, M.Ar, and T.Ar (p = 0.0094, 0.026, 0.026). Femurs from female mice treated with capsaicin were 6.0 and 2.3% shorter than femurs from female vehicle-treated mice at 4 and 8 weeks, respectively (p = 0.034, 0.0097). At 4 weeks, the femurs from female capsaicin-treated mice had 15% smaller M.Ar and 16% smaller T.Ar compared with vehicle-treated mice (p = 0.042, 0.031). Capsaicin treatment also affected cortical bone of the tibia. There were significant treatment*sex interactions for tibia M.Ar and T.Ar (p = 0.025, 0.0032). At 4 weeks, the tibias from female capsaicin-treated mice had 16% smaller M.Ar and 15% smaller T.Ar compared with vehicle-treated mice (p = 0.022, 0.029). In general, the magnitudes of observed structural changes were generally small.

*ELISA analysis of neuropeptides in bone (1e):*

Left tibias and femurs were collected from capsaicin- and vehicle-treated mice at the time of sacrifice, and were analyzed for neuropeptide concentrations using ELISA. Bones were analyzed for calcitonin-gene related peptide (CGRP) and substance P (SP). Bones were flash frozen with liquid nitrogen immediately following dissection, and were then crushed into small fragments, boiled in 2 M acetic acid in 4% EDTA (pH 3.5), and centrifuged at 3000 *g* for 15 min. Supernatants were freeze-dried and dissolved in ELISA buffer. Neuropeptide concentrations were determined using commercial mouse-specific ELISA assays (USCN Life Sciences, Inc., Wuhan, China), and data was normalized to the wet weight of the tibia to account for variation in bone size. Using this technique we were unable detect any effects of capsaicin treatment on concentrations of CGRP or SP in bone.

*ELISA analysis of serum biomarkers of bone turnover (1f):*

Blood was collected from capsaicin- and vehicle-treated mice immediately prior to sacrifice for quantification of systemic biomarkers of bone metabolism. Mice were anesthetized with isoflurane and approximately 100-200 μL of blood was collected retro-orbitally. Samples were allowed to clot for 2-4 hours and then centrifuged at 1000 g for 5 minutes. The supernatants were collected and frozen rapidly to -80 °C until analyzed. Serum was analyzed in duplicate to determine the concentrations of carboxy-terminal collagen crosslinks I (CTX-I) and

procollagen type 1 amino-terminal propeptide (P1NP) using commercial mouse-specific ELISAs (Cusabio, Wuhan, China) as per the manufacturer's instructions.

We did not detect a main effect of capsaicin treatment on serum concentrations of CTX-I or P1NP (Fig.5). Sex significantly affected serum concentrations of CTX-I ($p = 0.043$). Male capsaicin-treated mice at 8 weeks of age had 37% lower CTX-I concentrations than vehicle-treated male mice of the same age ($p = 0.046$). P1NP serum concentrations did not vary significantly by treatment, sex or age.

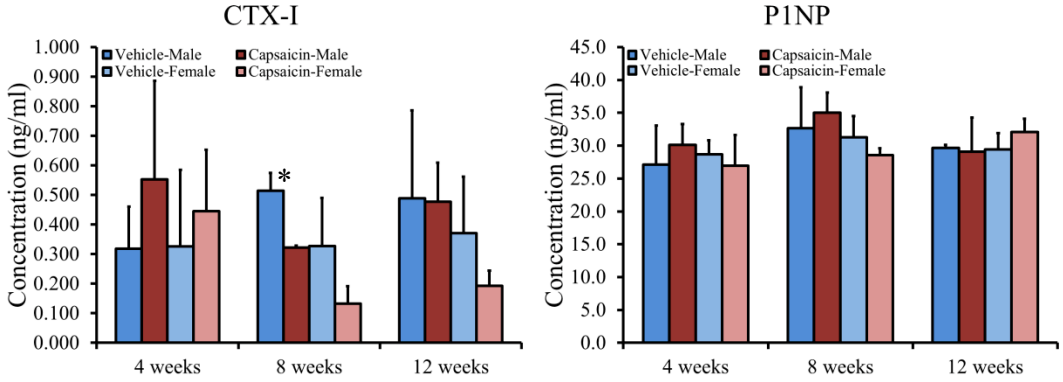


Fig. 3. Capsaicin treatment was not a main effect for serum concentrations of CTX-I or P1NP. * $p < 0.05$.

*Dynamic histomorphometry of fluorescent-labeled bone sections (1g):*

Mice received injections of calcein green (10 mg/kg; Sigma-Aldrich, St. Louis, MO) and Alizarin-3-methlimino-diacetic acid (30 mg/kg; Sigma-Aldrich, St. Louis, MO) 10 days and 3 days prior to sacrifice, respectively. After scanning with microCT, the right tibias were embedded in Technovit (Kulzer, Wehrheim, Germany) using standard techniques for undecalcified bone [12]. Two sections were cut from each bone on a bandsaw (Model 310, Exakt Technologies, Norderstedt, Germany) in the transverse plane at 40% of the length from the proximal end. The sections were ground to an approximate thickness of 40 μm. Two color fluorescent images were obtained at 10x magnification (Nikon Eclipse TE2000-E, Tokyo, Japan). Dynamic histomorphometric analysis was performed using commercial software (Bioquant, Nashville, TN) and the results were averaged for the replicate slides from each bone. We quantified mineral apposition rate (MAR), percent mineralizing surface (MS/BS), and bone formation rate (BFR) on the endosteal and periosteal surfaces.

Capsaicin treatment altered bone formation rate parameters in the tibias of treated mice (Table 2). There were significant treatment*sex*age interactions for MAR and BFR at the endosteal surface ($p = 0.023$, 0.020) and for MAR and MS/BS at the periosteal surface. For example, at 4 weeks of age, female capsaicin-treated mice had 28% higher MAR than female vehicle-treated mice at the endosteal surface of the tibia ($p = 0.042$). Figure 4 shows fluorescent images from female vehicle- and capsaicin-treated mice at 4 weeks. At 12 weeks, female mice treated with capsaicin had 23.4% lower MS/BS ($p = 0.012$) and a 22.4% trend toward lower BFR ($p = 0.061$) at the periosteal surface than female vehicle-treated mice.

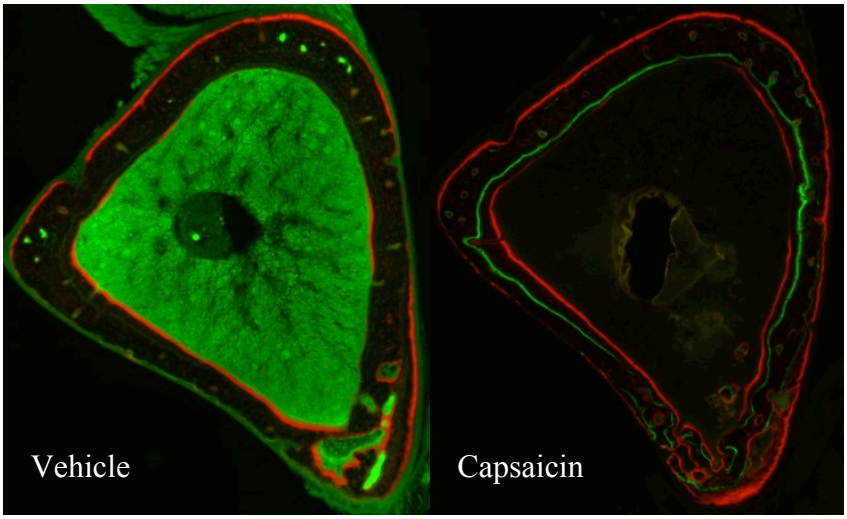


Fig. 4. Representative fluorescent images of cortical bone from the tibias of 4 week old, female vehicle- and capsaicin-treated mice, used for histomorphometric analysis. * $p < 0.05$.

| Table 2. Tibia Cortical Bone Histomorphometry | | | |
|---|---|---|---|
| | *MAR (μm/day)* | *MS/BS (%)* | *BFR ($μm^3$/ $μm^2$/day)* |
| *Endosteal Surface* | | | |
| **Vehicle-treated** | | | |
| 4 week | | | |
| M | 3.84 ± 0.53 | 76.5 ± 2.0 | 2.94 ± 0.47 |
| F | 3.12 ± 0.24 | 72.0 ± 9.9 | 2.23 ± 0.20 |
| 8 weeks | | | |
| M | 1.55 ± 0.01 | 65.0 ± 4.4 | 1.01 ± 0.07 |
| F | 2.38 ± 0.51 | 88.7 ± 7.9 | 2.10 ± 0.46 |
| 12 weeks | | | |
| M | 1.33 ± 0.30 | 88.1 ± 5.7 | 1.18 ± 0.32 |
| F | 1.56 ± 0.48 | 78.8 ± 5.9 | 1.24 ± 0.48 |
| **Capsaicin-treated** | | | |
| 4 weeks | | | |
| M | 3.56 ± 1.18 | 74.0 ± 2.3 | 2.64 ± 0.94 |
| F | 4.35 ± 0.74[a] | 80.0 ± 12 | 3.51 ± 1.00 |
| 8 weeks | | | |
| M | 2.09 ± 0.73 | 69.9 ± 13 | 1.51 ± 0.79 |
| F | 1.95 ± 0.05 | 80.2 ± 6.7 | 1.56 ± 0.11 |
| 12 weeks | | | |
| M | 1.47 ± 0.16 | 78.9 ± 16 | 1.17 ± 0.37 |
| F | 1.59 ± 0.23 | 86.7 ± 5.0 | 1.38 ± 0.25 |
| | | | |
| *Periosteal Surface* | | | |
| **Vehicle-treated** | | | |
| 4 weeks: | | | |
| M | 3.81 ± 0.82 | 64.8 ± 12 | 2.44 ± 0.53 |
| F | 2.93 ± 0.48 | 61.9 ± 18 | 1.76 ± 0.32 |
| 8 weeks | | | |
| M | 1.41 ± 0.13 | 57.6 ± 17 | 0.80 ± 0.17 |
| F | 1.77 ± 0.14 | 46.2 ± 6.3 | 0.80 ± 0.25 |
| 12 weeks | | | |
| M | 0.97 ± 0.12 | 33.5 ± 5.7 | 0.33 ± 0.09 |
| F | 1.05 ± 0.11 | 46.0 ± 3.7 | 0.49 ± 0.07 |
| **Capsaicin-treated** | | | |
| 4 weeks | | | |
| M | 3.25 ± 0.25 | 64.3 ± 16 | 2.07 ± 0.43 |
| F | 3.26 ± 0.31 | 54.6 ± 12 | 1.77 ± 0.35 |
| 8 weeks | | | |
| M | 2.07 ± 0.74 | 39.8 ± 9.0 | 0.79 ± 0.11 |
| F | 1.68 ± 0.15 | 50.0 ± 10 | 0.84 ± 0.19 |
| 12 weeks | | | |
| M | 1.50 ± 1.28 | 48.8 ± 16 | 0.75 ± 0.37 |
| F | 1.07 ± 0.11 | 35.2 ± 4.8[a] | 0.38 ± 0.06 |
| Histomorphometry results from assessment of tibia cortical bone. All data are expressed as mean ± SD.<br>[a] Significant difference between capsaicin and vehicle-treated mice, $p < 0.05$. | | | |

*Three-point bending mechanical testing of mouse radii:*

Bilateral radii were removed post mortem and preserved in 70% ethanol. Bones were scanned using micro-computed tomography as described above, with a volume of interest that included the entire bone. Average bending moment of inertia for the central 1.0 mm (100 slices) was determined using BoneJ analysis of the microCT images [13]. Following μCT, radii were mechanically tested in three-point bending to determine cortical bone material properties. The lower supports had a span of 5.02 for 4 week samples, and a span of 7.45 mm for 8 and 12 week samples, and the center loading platen was driven at 0.2 mm/sec until failure.

Resulting force and displacement data were analyzed to determine stiffness and yield force. Modulus of elasticity and yield stress were determined using Euler-Bernoulli beam theory.

Capsaicin treatment significantly decreased the mechanical properties of radii tested with three-point bending. There was a main effect of capsaicin treatment on yield force and moment of inertia ($p = 0.047$, 0.0041). At 12 weeks, radii of mice treated with capsaicin yielded at 9.9% lower force compared with vehicle-treated mice ($p = 0.0067$). Radii of male mice treated with capsaicin had 9.5% lower moment of inertia than vehicle-treated male mice at 12 weeks ($p = 0.028$). Calculations of the yield stress and modulus of elasticity revealed no significant differences between capsaicin- and vehicle-treated mice.

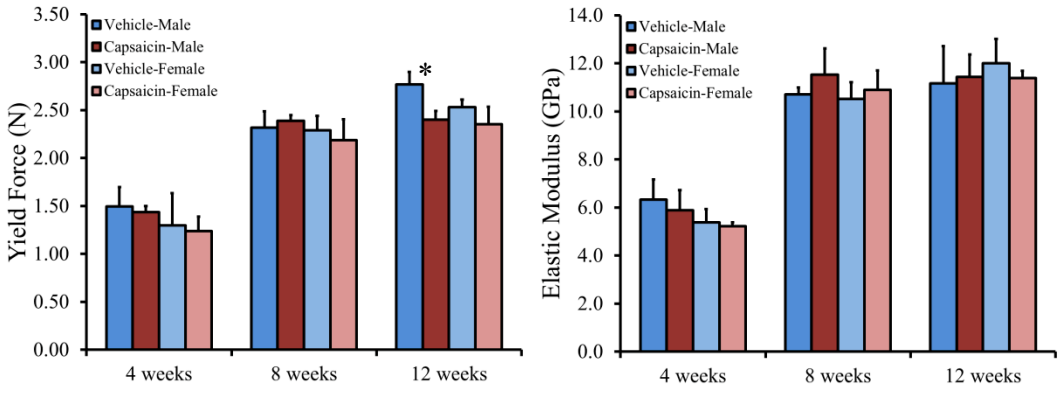


Fig. 5. Capsaicin treatment significantly affected yield force but not elastic modulus of radii tested in three-point bending. * $p < 0.05$.

**KEY RESEARCH ACCOMPLISHMENTS:**

- Established neonatal capsaicin treatment model of peripheral sensory nerve degeneration in our laboratory
- Confirmed that neonatal capsaicin treatment decreases thermal sensitivity (peripheral sensory nerve function) in C57BL/6 mice, lasting until at least 12 weeks of age
- Quantified trabecular and cortical bone structure in capsaicin- and vehicle-treated mice at 4, 8, and 12 weeks of age using micro-computed tomography, yielding a small-magnitude but statistically significant bone phenotype in capsaicin-treated mice
- Analyzed neuropeptides in bone and serum biomarkers of bone turnover using ELISA in capsaicin- and vehicle-treated mice
- Directly measured bone formation rates in capsaicin- and vehicle-treated mice using fluorescent-labeled dynamic histomorphometry
- Quantified bone mechanical properties in capsaicin- and vehicle-treated mice using 3-point bending of radii
- Currently conducting strain gage analysis of bone strain during tibial compression in order to normalize bone strain magnitudes for Aim 2
- Currently conducting neonatal capsaicin and vehicle treatment for Aim 2 mice

**REPORTABLE OUTCOMES:**

- Presented preliminary research in oral presentation at the Orthopaedic Research Society 2013 Annual Meeting
- Manuscript encompassing Aim 1 – to be submitted to the Journal of Musculoskeletal and Neuronal Interaction by the end of the calendar year
- Application for funding - Ruth L. Kirschstein National Research Service Awards for Individual Predoctoral Fellows (F31) for Mollie Heffner – submitted August, 2013
- Application for funding - NIH Exploratory Developmental Research Grant Program (R21) – to be submitted February, 2014

**CONCLUSION:** This research investigates mechanisms by which peripheral sensory nerves influence bone maintenance and mechanotransduction using capsaicin-injected mice as a model of decreased peripheral sensory nerve function. In **Aim 1** we quantified the relationship between reduced peripheral sensory nerve function and bone structure and mechanical properties. We hypothesized a negative correlation between reduced sensory nerve function and structure and mechanical properties of bone. Consistent with this

hypothesis, we observed a small but statistically significant decrease in trabecular and cortical bone structure due to capsaicin treatment. However, it is unclear whether this change in bone structure/properties is meaningful for fracture susceptibility in bone. It is possible that the phenotype of capsaicin-treated mice will be exhibit the most meaningful differences when challenged by increased mechanical loading. To this end, we are pursuing Aim 2. In **Aim 2** we will determine the bone adaptation response of capsaicin- and vehicle-treated mice to increased mechanical loading (tibial compression). We hypothesize that capsaicin-treated mice will have an *increased bone adaptation response* to mechanical loading due to decreased negative feedback by peripheral sensory nerves. We further hypothesize that concentrations of biomarkers of bone metabolism will be lower and change less with loading in capsaicin-injected mice. Altogether, this research will establish the role of peripheral nerves in age-related decreases in bone's ability to adapt to exercise. These studies may lead to novel therapies aimed at preserving healthy bone turnover with age. This research will be the basis for future studies investigating the interaction of peripheral nerves and bone, and peripheral nerve function as a potential mechanism of age-related bone loss.

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